Patient A.S. (UPN 26) Allo-BMT - 8.11.1994				Patient M.L. (UPN 45) Allo-BMT - 15.11.1996					Patient E. M. (UPN 18) Allo-BMT - 24.08.1993			
CML	FISH Bcr-abl (+)	RT-PCR	Chimerism*	CML	FISH Bcr-abl (+)	RT-PCR	Chimerism*	FISH X, Y	CML	FISH Bcr-abl (+)	RT-PCR	Chimerism*
Relapse 11.03.99				Relapse 07.10.98					Relapse 01.02.00			
16.03.99	50%		Recipient's genotype	20.10.98			60-80% R		08.02.00	9,9 %		Mixed
12-24.03.99 HU				21.10.98 DLI I					29.02.00	23,1%		Recipient's genotype
24.03.99 -11.01.00 INF-alfa				HU 7.10-5.11.98					I DLI 14.03.00			Mixed
28.10.99	2,92%	b3a2	100 % D	INF-alfa 02.11.- 02.12.98					12.04.00	37,3 %	b3a 2	Mixed
04.11.99		b3a2		18.01.99		b2a2	60-80 % R		26.04.00	51,3%		
16.02.00	2%			01.03.99		b2a2	20-40 % R		II DLI 05.05.00			
14.06.00			100 % D	23.03.99		b2a2	10-20 % R		06.06.00	2,2%	b3a 2	Mixed
27.06.00			100 % D	29.03.99	2%	b2a2	100 % D		04.07.00		b3a 2b2 a2	100 % D
26.07.00			100 % D	19.04.99 21.04.99	20% 23%	b2a2	100% D	23% XX 77% XY	10.08.00			100 % D
I DLI 28.06.00			100 % D	05.07.99	17%	b2a2	100 % D		14.11.00		b2a 2	100 % D
30.08.00	3%		100 % D	22.09.99	6%	b2a2	100 % D	8% XX	10.01.01		b3a 2b2 a2	
07.12.00	0%	b2a2	100 % D	28.09.99		b2a2			07.02.01		b3a 2	
25.04.01	22%	b2a2		22.12.99	5%	b2a2			09.04.01		-	
26.06.01	10%	b2a2		29.03.00	1,97 %	b2a2	100 % D		27.06.01		-	100 % D
28.08.01	21%	b2a2	100 % D	06.06.00		b2a2	100 % D					
29.09.01	10%	b2a2		06.09.00	0%	-	100 % D	100% XY				
				30.11.00		b2a2	100 % D					
				03.01.00		-	100 % D					
				22.03.01	0%	b2a2	100 % D	100% XY				
				12.09.01	7%	b2a2	100 % D					
				09.11.01	23%	b2a2	100 % D	77% XY				

* examinations of chimerism status with the use of STR-PCR were performed without recipient's control sample before BMT.

Conclusion: Combined evaluation of the minimal residual disease by FISH, RT-PCR and hematopoietic chimerism by STR-PCR method gives more informative details concerning response to adoptive immunotherapy in post-transplant relapsed CML patients with BCR/ABL fusion gene.

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9.
RELAPSE AFTER HSCT IS RELATED TO HIGHER IN VITRO RESISTANCE TO MOST DRUGS EXCEPT FOR TREOSULFAN AND ETOPOSIDE

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Background and Objective: Results of hematopoietic stem cell transplantation (HSCT) in childhood acute leukemias, are still unsatisfactory, however better than obtained with conventional chemotherapy. Probability of long-term survival after HSCT is still only 40% - 50%. The main cause of failure remains relapse of the disease. Current possibilities to augment an antileukemic effect of HSCT include: protection of GvHD and introduction of adoptive immunotherapy combined with the results of molecular chimerism and minimal residual disease. Another possibility is the use of more intensive or tailored preparative regimen (prep-reg) before HSCT and modulation of drug resistance of residual leukemic cells against agents used in prep-reg. The aim of the study is the analysis of in vitro drug resistance profile in aspect of leukemia relapse after HSCT.

Patients and Methods: A total number of 22 children with acute leukemia (14 ALL, 8 AML), aged 1,9-17 years, who underwent HSCT, were included into the study. For all children, drug resistance profile was done by the MTT assay. Leukemic cells of each child were tested for cytotoxicity of up to 26 drugs (busulfan and melfalan was not tested). Children who have died due to peritransplant complications were excluded from the study. In 13 children HSCT was performed from HLA identical sibling, in 2 from MUD, in 1 from MMRD and 6 children had autotransplantation. 8/22 children relapsed after HSCT. Drug resistance profile was performed in Laboratory of Clinical and Experimental Oncology in Bydgoszcz. The cytotoxicity was expressed as the concentration of drug, which was lethal to 50% of tested blasts. Children with ALL were prepared to HSCT with: FTBI/VP±CY (6 patients) or BU/VP/CY (8 patients). Before MUD-HSCT, ATG was also given. AML children were conditioned with one the following sets of drugs: BU/VP/CY, BU/CY/MEL, BU/CY, BU/MEL, TREO/VP/CY, TREO/FLU/ATG, TRE/FLU/MEL.

Results: Children who relapsed after HSCT showed higher in vitro resistance of leukemic blasts to most of tested drugs including cyclophosphamide (3,3-fold) and fludarabine (2,3-fold). Only for 4/26 tested drugs chemosensitivity of relapsed patients was better, i.e.: to treosulfan (4,8-fold), etoposide (1,7-fold), thiotepa (4,6-fold) and mercaptopurine (1,7-fold) (all differences did not reach statistical significance). 5/6 children prepared with FTBI stays in remission.

Conclusions: The results of this analysis might suggest that relative sensitivity of leukemic blasts to treosulfan and etoposide is not sufficient to prevent patient from relapse after HSCT. This study give an advantage for the use of cyclophosphamide and FTBI in pre-reg, however this observations require further investigations.

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10.
TREOSULFAN WITH FLUDARABINE AND MELPHALAN AS CONDITIONING REGIMEN FOR SECOND ALLOGENEIC BMT IN A CHILD WITH POST-TRANSPLANT MDS RELAPSE RESISTENT TO ADOPTIVE IMMUNOTHERAPY – A CASE REPORT

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We report a 10-year old girl with a diagnosis of myelodysplastic syndrome, who after first bone marrow transplantation (HLA –matched sibling donor - May 1999) suffered from relapse (Dec 2001) and subsequently was treated by donor lymphocyte infusions (twice) with no response. We decided to perform a second transplantation from the same donor but with the use of different conditioning regimen. It consisted of: treosulfan (10 g/m^2/day for 3 days, fludarabine (30 mg/kg/day for 5 days) and melphalan (140 mg/kg/day single dose). On day ,,0,, (30 May 2001) she was infused with bone marrow from her 8-year old, HLA-identical